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# Solid Lipid Nanoparticles Incorporating Melatonin as New Model for Sustained Oral and Transdermal Delivery Systems

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**Introduction:** melatonin (MT) is a hormone produced by the pineal gland at night, involved in the regulation of circadian rhythms. For clinical purposes, exogenous MT administration should mimic the typical nocturnal endogenous MT levels, but its pharmacokinetics is not favourable due to short half-life of elimination. Aim of this study is to examine pharmacokinetics of MT incorporated in solid lipid nanoparticles (SLN), administered by oral and transdermal route. SLN peculiarity consists in the possibility of acting as a reservoir, permitting a constant and prolonged release of the drugs included. In 7 healthy subjects SLN incorporating MT 3 mg (MT-SLN-O) were orally administered at 8.30 a.m. MT 3 mg in standard formulation (MT-S) was then administered to the same subjects after one week at 8.30 a.m. as controls. In 10 healthy subjects SLN incorporating MT were administered transdermally (MT-SLN-TD) by the application of a patch at 8.30 a.m. for 24 hours. Compared to MT-S, T<sub>max</sub> after MT-SLN-O administration resulted delayed of about 20 minutes, while mean AUC and mean half life of elimination was significantly higher (respectively  $169944.7 \pm 64954.4$  pg/ml  $\times$  hour vs.  $85148.4 \pm 50642.6$  pg/ml  $\times$  hour,  $p = 0.018$  and  $93.1 \pm 37.1$  min vs.  $48.2 \pm 8.9$  min,  $p = 0.009$ ). MT absorption and elimination after MT-SLN-TD demonstrated to be slow (mean half life of absorption:  $5.3 \pm 1.3$  hours; mean half life of elimination:  $24.6 \pm 12.0$  hours), so MT plasma levels above 50 pg/ml were maintained for at least 24 hours. This study demonstrates a significant absorption of MT incorporated in SLN, with detectable plasma level achieved for several hours in particular after transdermal administration. As dosages and concentrations of drugs included in SLN can be varied, different plasma level profile could be obtained, so disclosing new possibilities for sustained delivery systems.

**Keywords:** Solid Lipid Nanoparticles, Melatonin, Transdermal.

## 1. INTRODUCTION

Melatonin (*N*-acetyl-5methoxytryptamine, MT) is a hormone produced by the pineal gland at night. It is involved in the regulation of sleep and circadian rhythms, working as a time-of-day information to various organs.<sup>1,2</sup> The synthesis and secretion of MT is induced by darkness and suppressed by light through retinal nerve fibers projecting to the suprachiasmatic nucleus of hypothalamus, then to the superior cervical ganglion and finally to the pineal

gland.<sup>3</sup> Plasma MT levels are low during the day, about 10 pg/ml in young adults, with a pulse of secretion starting at 9 p.m. and reaching a peak of about 70–100 pg/ml between 2 and 4 a.m. Plasma MT levels then return to baseline at 7–9 a.m.<sup>4</sup> With aging and in healthy individuals with insomnia nocturnal peak level of MT is reported to be lower and delayed of about one hour compared to young adults with good sleep quality.<sup>5,6</sup> MT has proved to be useful particularly in disorders of sleep-wake cycle and in insomnia of the elderly.<sup>7</sup> Nevertheless, no doses or exact timing of administration have yet been agreed for any condition, due to inconstancy of clinical results, variability

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of pharmacokinetic studies and lack of data comparing the efficacy of various formulations. For clinical purposes, exogenous MT administration should be able to mimic the typical nocturnal endogenous MT levels, but its pharmacokinetics is not favourable. After an intravenous bolus injection MT half-life of elimination is very short (about 40 min)<sup>8</sup> and, when administered orally, MT shows low bioavailability and a rapid clearance from plasma influenced by a marked first pass hepatic metabolism.<sup>9</sup> Over 80% of MT is then excreted in the urine as 6-SMT. Therefore MT is not a good candidate for conventional oral immediate release system and a sustained release delivery system is required in order to maintain prolonged plasma MT concentrations. Transdermal delivery systems of MT have been described by several authors.<sup>10–12</sup> They should have the advantage of avoiding first-pass metabolism but may not be practical for particular purposes.

Aim of this study is to examine in voluntary healthy subjects the release and pharmacokinetics of MT incorporated in solid lipid nanoparticles (SLN), administered orally and by transdermal route, after dispersion in an expressly-studied vehicle. The peculiarity of these two new formulations is the use of SLN prepared from warm microemulsions. SLN can incorporate hydrophilic and lipophilic drugs, work as a reservoir for sustained release of them and are targeted to lymph after duodenal administration.<sup>13–15</sup> Moreover stealth-SLN can be prepared to avoid their recognition by reticuloendothelial system.<sup>16,17</sup> SLN have been successfully administered to laboratory animals via duodenal,<sup>15</sup> parenteral,<sup>18</sup> and ocular routes.<sup>19</sup> MT incorporated in SLN (MT-SLN) has been chosen for our *in vivo* study because of the safeness of MT administration in humans even at high dosages.<sup>7</sup> Our study intends to furnish a general model for sustained release delivery systems of drugs devoid of favourable pharmacokinetics and requiring therapeutic plasma levels lasting for several hours.

## 2. MATERIALS AND METHODS

### 2.1. MT Incorporated in SLN (MT-SLN)

#### 2.1.1. Components

Melatonin was a kind gift by Helsinn (Biasca, Switzerland), stearic acid was from Merck, Epikuron 200 (Soya phosphatidylcholine 95%) was a kind gift from Degussa (Hamburg, Germany), taurocholate sodium salt was a kind gift from PCA (Basaluzzo, Italy). The other chemicals were of analytical grade.

#### 2.1.2. Preparation of SLN

Melatonin was added to a mixture of stearic acid and Epikuron 200, melted at about 70 °C. Successively a solution of sodium taurocholate in water, heated at the same temperature, was added. Using the right amounts of the components clear warm microemulsions were

achieved. SLN were then obtained by dispersing the warm microemulsion in cold water (2–3°). The dispersion was successively washed three times by ultrafiltration (Vivaflow 50, cut-off 100.000 Dalton). The washed suspension was then freeze-dried (Criolab 8—Crioforma—Torino, Italy) in the presence of 2% (p/v) of lactose (Pharmatose DCL11, DMW international). Average sizes and polydispersity indices of MT-SLN were measured by photocorrelation spectroscopy (Zetasizer—3000 HS—Malvern Instruments, UK). The analysis of percentages of melatonin incorporated in SLN was performed by a reversed HPLC method (Jasco pump-2080; Jasco Detector UV-2080). The mobile phase was water–methanol (50:50). The analysis was performed at a flow rate of 1 ml/min at 229 nm. A weighed amount of freeze-dried MT-SLN at first was dissolved in methanol. An aliquot, dissolved in the mobile phase, was then employed.

#### 2.1.3. Thickened Gel Preparation

To 2 g of a mixture (w/w) water propylene glycol (70:30), 200 mg of MT-SLN (MT = 1.8%) was kindly mixed and successively under stirring Carbopol 940® (1.20%) was added, obtaining a gel. Vertical Franz cells were used: the full-thickness dorsal skin of male hairless mice (athymic-nude-Charles River) aged 4–5 weeks was used. The skin previously rinsed with normal saline was sandwiched between the two ground panels. The gel (0.3 g) was gently applied to the donor site of the skin surface, which had an available diffusion area of 1.6 cm.<sup>2</sup> The content of the receptor cell was continuously stirred and thermostated at 25°. At appropriate intervals the entire contents of the receptor chamber was removed by HPLC determination and the cell refilled with fresh aqueous solution at pH 6.0. The experiments were performed in triplicate.

#### 2.1.4. Transdermal Preparation

Freeze-dried MT-SLN (MT = 2%) were dispersed in a mixture of water and propylene glycol (70:30)(w/w) and successively added of Carbopol 940® (1.2%) obtaining a gel (MT-SLN-TD); the melatonin concentration in the gel was 1.8 mg/g (w/w).

#### 2.1.5. Oral Preparation

Freeze dried amounts of MT-SLN (MT = 4.13%) containing 3 mg MT were included in hard gelatine capsules (Scherer) for oral administration (MT-SLN-O); 3 mg of MT was also included in hard-gelatine capsules for oral administration (MT-S).

## 2.2. MT Administration and Subjects

The subjects enrolled were healthy volunteers. They gave informed consent and the experiment was approved by the

local ethical committee. Inclusion criteria were: absence of sleep disorders, excessive daytime somnolence or disorders of sleep-wake cycle, absence of known diseases involving central nervous system or endocrine system, absence of any medication treatment at least one month before the study. All subjects maintained a regular sleep-cycle during the week of the study.

In 7 subjects (4 males, 3 females; mean age:  $43.8 \pm 9.3$  years) one capsule of MT-SLN-O was orally administered at 8.30 a.m. One capsule of MT-S was administered to the same subjects after one week at 8.30 a.m. The days of oral administrations subjects abstained from eating in the morning in order to prevent possible interferences with absorption. Blood samples were collected at regular intervals: every 30 minutes until 2nd hour after administration, every hour until 8th hour, and finally at 24th hour.

In 10 subjects (5 males, 5 females, mean age  $48.2 \pm 16.3$  years) 2 g of MT-SLN-TD (corresponding to melatonin 3.6 mg, 0.4 mg MT/cm<sup>2</sup>) was applied at 8.30 a.m. to 9 cm<sup>2</sup> skin area over the anterior part of the chest, delimited by 1 mm thick biocompatible foam tapes and covered by a polyester-based membrane (3M<sup>®</sup> Scotchpak<sup>®</sup>) and an occlusive membrane (Smith and Nephew<sup>®</sup> OpSite Flexigrid<sup>®</sup>) in order to prevent evaporation of some components. In these conditions a single layer of MT-SLN (1 mm thick) was directly in contact with skin surface and acted as a reservoir of MT for transdermal absorption. MT-SLN-TD patch was then removed after 24 hour. The skin was not pre-treated. In all subjects blood samples were collected at 8 a.m., half an hour after administration, then every 2 hours until 11th hour after administration. In two subjects blood samples were collected at 8 a.m., half an hour after administration, every 2 hours until 11th hour. In the following 2 days, the blood samples were collected every 3–4 hours from 8 a.m. to 7 p.m. on 2nd day and from 8 a.m. to 3 p.m. on 3rd day. The temporary interruption of blood collection corresponds to night time and the sleep period. As baseline controls, in this group of subjects blood samples were collected after one week and at the same intervals from 8 a.m. until 7 p.m.

### 2.3. MT Assays in Serum

Blood was centrifuged, immediately separated and stored at  $-20^{\circ}\text{C}$  until assayed. Serum MT levels were determined after diethyl ether extraction using radioimmunoassay (RIA) method. All samples from an individual subject were analyzed in the same kit. The assay sensitive was 2 pg/ml. Duplicate MT determinations were made from each samples. The intra-assay coefficients of variation (CV) ranged from 1 to 5%. The intra-assay CV was 8.1%.

### 2.4. Pharmacokinetic Analysis

Plasma drug concentration data of the individual subjects were analyzed by standard noncompartmental methods.

The individual peak concentrations (C<sub>max</sub>) and times to C<sub>max</sub> (T<sub>max</sub>) were obtained. The area under the curve (AUC) to the last measurable concentration was calculated with the log-linear trapezoidal rule, and the absorption and elimination half-life determined. The data were statistically analysed using Prism 3.0<sup>®</sup> (GraphPad Software<sup>®</sup>) and Sigma Plot for Windows<sup>®</sup> version 8.0. Two-tailed unpaired Student's t test or 2-way ANOVA and Post Hoc tests were used when appropriate to compare different administrations of MT. The pharmacokinetic data obtained in two subjects after MT-SLN-TD administration and followed for 3 days were fitted with the function  $y = \text{span } x (e^{-K_1x} - e^{-K_2x})$ .

## 3. RESULTS

### 3.1. MTSLN Characterization

Three kind of freeze-dried MT-SLN containing different amounts of melatonin were prepared and characterized: (a) MT-SLN: MT = 1.8% for *in vitro* experiments (average diameter: 85 nm, polydispersity index: 0.135); (b) MT-SLN: MT = 2% for transdermal application (average diameter: 91 nm, polydispersity index: 0.140); (c) MT-SLN: MT = 4.13% for oral route (average diameter: 111 nm, polydispersity index: 0.189)

### 3.2. In Vitro Release of Melatonin from Gel

A flux of melatonin of 31 ug/h/cm<sup>2</sup> through hairless mice skin was obtained. A pseudo-zero order kinetics was followed.

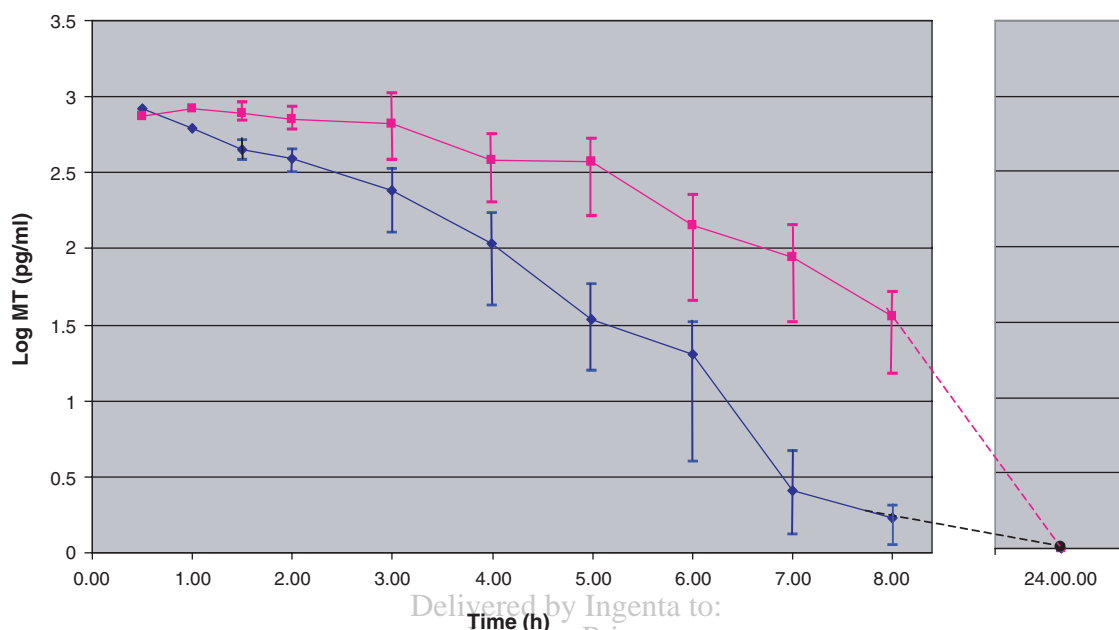
### 3.3. Pharmacokinetic Analysis After Oral Administration

Pharmacokinetic analysis after MT-S and MT-SLN-O administration is shown in Table I. Figure 1 shows the profiles of MT plasma levels after MT-SLN-O administration compared to MT-S. MT absorption demonstrated to

**Table I.** Pharmacokinetic analysis after MT-S and MT-SLN-O administration.

	Mean	SD	Min	Max
MT-S				
C <sub>max</sub>	831.6	407.7	445.0	1097.0
T <sub>max</sub>	30	—	30	30
AUC	85148.4	50642.6	528.91	826.00
Half-life of elimination	48.2	8.9	38.3	66.3
MT-SLN-O				
C <sub>max</sub>	828.4	307.6	421.0	1085.0
T <sub>max</sub>	51.4	14.6	30	60
AUC	169944.7	64954.4	528.91	826.00
Half-life of elimination	93.1	37.1	59.6	155.6

\**p* = 0.009, °*p* = 0.018, MT-S versus MT-SLN-O (2-tailed unpaired student's t test).

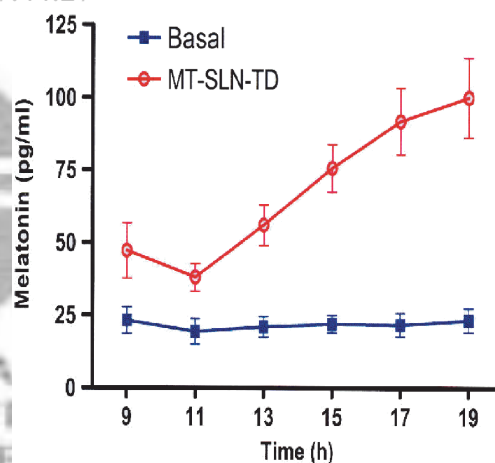


**Fig. 1.** MT plasma level profiles after MT-S (◆) and MT-SLN-O (■) administration.

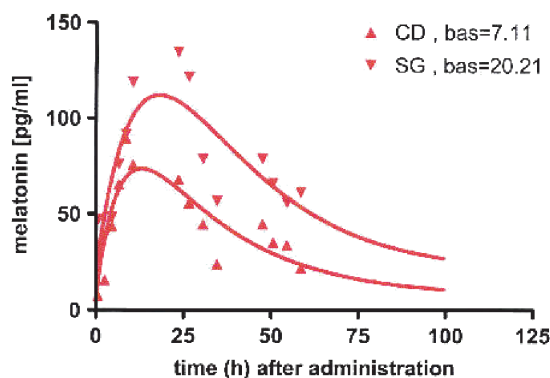
be rapid as in all subjects MT plasma levels higher than 200 pg/ml were detected in blood samples after 30 minutes from administration of both MT-S and MT-SLN-O. Mean  $C_{max}$  after MT-S and MT-SLN-O administration were comparable and did not show statistical differences ( $831.6 \pm 407.7$  pg/ml vs.  $828.4 \pm 307.6$  pg/ml).  $T_{max}$  after MT-SLN-O administration resulted delayed of about 20 minutes compared to MT-S. However, mean half life of elimination of the drug after MT-SLN-O administration was significantly higher compared to MT-S ( $93.1 \pm 37.1$  min vs.  $48.2 \pm 8.9$  min;  $p = 0.009$ ) and, more interestingly, mean AUC after MT-SLN-O administration was considerably higher compared to MT-S ( $169944.7 \pm 64954.4$  pg/ml  $\times$  hour vs.  $85148.4 \pm 50642.6$  pg/ml  $\times$  hour;  $p = 0.018$ ). MT plasma level above 50 pg/ml was found in 5 subjects and 2 subjects, respectively 6 hours and 8 hours after MT-SLN-O administration. On the contrary only one subject presented MT plasma level above 50 pg/ml, 5 hours after MT-S administration, and none after 7 hours. No relationship between age or sex and MT plasma levels could be evidenced using both formulations. Mild somnolence was referred by 3 subjects after  $3.2 \pm 1.2$  hours from MT-SLN-O administration and lasted for a maximum of 2 hours. No subjects presented significant side effects.

### 3.4. Pharmacokinetic Analysis After Transdermal Administration

Figure 2 shows mean MT plasma level profiles at baseline and after MT-SLN-TD administration including all the subjects. Table II and Figure 3 shows pharmacokinetic analysis and MT plasma level profile after MT-SLN-TD



**Fig. 2.** MT plasma level profile at baseline and after MT-SLN-TD administration (10 subjects).



**Fig. 3.** MT plasma levels and fit function  $y = \text{span} * (e^{-K1x} - e^{-K2x})$  after MT-SLN-TD administration (2 subjects: CD and SG).

**Table II.** Pharmacokinetic analysis after MT-SLN-TD administration (2 subjects: CD and SG). Fit function:  $y = \text{span} * (e^{-K1x} - e^{-K2x}) + \text{base-line}$  (mean of control values). Cmax and AUC do not include baseline.

	CD	SG
SPAN	147.5	536.4
K1	0.03731	0.04326
K2	0.138	0.06913
Half life of absorption (hour)	18.57411	16.01942
Half life of elimination (hour)	5.021739	10.02459
AUC (pg/ml $\times$ hour)	2884.523	4640.151
Tmax (hour)	12.99029	18.11984
Cmax (pg/ml)	66.28431	91.66227

administration, together with the fit of function  $y = \text{span} * (e^{-K1x} - e^{-K2x})$ , considering only the two subjects who underwent blood sample collection until 54th hour. MT absorption demonstrated to be slow. A significant increase of MT plasma level was detected 4.5 hours after patch application and Cmax was lower than peak plasma levels obtained after MT-SLN-O administration. On the contrary Tmax and half life of elimination resulted much longer than oral administration, with K2 (elimination) higher than K1 (absorption). Clearly nocturnal endogenous MT plasma concentration overlies exogenous MT during nighttime and early morning, but after 9 a.m. and during daytime the contribution of endogenous MT is lower than 20 pg/ml. So, mean MT plasma level at 8 a.m. and 9 a.m. resulted higher compared to MT plasma levels detected 2.5 hours after administration, but this difference was not statistically significant according to post-hoc tests. As shown in Figures 2 and 3 mean MT plasma levels resulted significantly higher compared to baseline and above 50 pg/ml from 4.5 hours until at least 24 hours after MT-SLN-TD administration. No adverse effect was referred apart from a mild transient erythema on the site of patch application.

#### 4. DISCUSSION

The pseudozero order kinetics of melatonin included in SLN and released through hairless mice skin aimed us to study the *in vivo* release in healthy subjects. Significant absorption and detectable plasma level of MT was found in all subjects after oral and transdermal administration. These data suggest that also *in vivo* a significant release of drugs from SLN may be achieved. Indeed only 0.4 mg of melatonin in SLN/cm<sup>2</sup> was able to perform interesting amounts of MT for more than 24 hours.

Compared to standard oral formulation, MT-SLN-O administration demonstrated to obtain comparable peak plasma levels of the drug, even if delayed of about half an hour. More interestingly, detectable and clinically significant MT plasma levels after MT-SLN-O administration were maintained for a longer period of time. This means that this new formulation is able to give a sustained release of the drug that could be useful for those substances

devoid of favourable kinetics such as MT. In previous studies<sup>14, 15</sup> the targeting to lymph of SLN, both drug-loaded or unloaded, was proved after duodenal administration in laboratory animals. Probably, also if the capsules containing SLN were not gastro-resistant, MT-SLN may partly be targeted to lymph allowing longer half life of the molecule *in vivo*. In fact MT half-life of elimination is about 40 min after an intravenous bolus and when it is administered orally low bioavailability and rapid clearance from plasma is seen, primarily due to a marked first pass hepatic metabolism.<sup>8, 9</sup> In our study mean half life of elimination is about doubled compared to these data regarding intravenous administration and compared to standard formulation we used as control.

Nevertheless some differences among subjects were found and deserve further investigation in order to analyse all the variables involved in oral absorption *in vivo*. In our study high MT plasma levels were detected in all subjects after about one hour, and MT plasma level above 50 pg/ml after 8 hours was found in 2 subjects after MT-SLN-O administration. This means that MT absorption is relatively rapid initially, and present little difference among subjects. On the contrary subjects may differ according to the capacity of SLN to work as a reservoir for the drug, or they may differ according to the rapidity of drug clearance from plasma. Our preliminary data are in accordance with the coexistence of both these hypotheses. Only one subject presented prolonged relatively high MT levels both after MT-S and MT-SLN-O administration, so a low clearance from plasma may be hypothesized. In all other subjects this relation was not found, so different amount of drug released over time by SLN may explain our data.

More interesting, pharmacokinetic analysis regarding MT-SLN-TD administration demonstrates that plasma level of the drug comparable to oral administration may be achieved for more than 24 hours. These data are very encouraging, and represent a new model of a transdermal sustained release of drugs devoid of favourable pharmacokinetics. Transdermal drug delivery systems offer several advantages over the parental and oral routes as they may avoid the patient's unwillingness or incapability to swallow oral preparations and may prevent issues associated with hepatic first-pass metabolism, poor absorption from the gastrointestinal tract and variable bioavailability.

In recent years technologies for the development of transdermal drug delivery systems have shown great progresses and now more molecules for therapeutic use can be included in media for transdermal absorption.<sup>20, 21</sup>

Our model is based on new pharmaceutical technology that uses SLN as a reservoir and a carrier of the drug. The long half life of absorption makes this formulation not suitable for drugs requiring a rapid increase of plasma levels. On the contrary this preparation might be able to give sustained release of the drugs for several h, according to the amount of the drug included in SLN and the application areas.

We have chosen to include melatonin into SLN because of its low incidence of adverse effects even at high dosages.<sup>7</sup> Moreover the components of SLN are biocompatible. In our study tolerability of MT-SLN-TD and MT-SLN-O was good and no adverse effect occurred apart from predictable mild somnolence and transient erythema after gel application. This means that, at least at dosages used in our study, SLN administration via oral and transdermal route is safe. Further studies in a larger group of subjects and after prolonged treatments are needed in order to verify tolerability and long-term possible adverse effects. Nevertheless our study indicates that MT-SLN-O and MT-SLN-TD may be considered models for new sustained delivery systems *in vivo* that could be generalized to other substances, in particular to those medications requiring prolonged high plasma levels despite their unfavourable pharmacokinetics. As dosages and concentrations of drugs included in SLN can be varied, different plasma level profile could be obtained, so disclosing new possibilities for sustained delivery systems. Further studied will be directed to optimize dosages and concentration of the drugs included in SLN, and to verify the adequate application areas over the skin for transdermal delivery systems.

## References and Notes

1. R. J. Reiter, *Endocr. Rev.* 12, 151 (1991).
2. F. Fraschini and B. Stankov, *Chronobiologia* 21, 89 (1994).
3. J. Barrenetxe, P. Delagrangé, and J. A. Martínez, *J. Physiol. Biochem.* 60, 61 (2004).
4. J. Arendt, *Chronobiol. Int.* 23, 21 (2006).
5. F. Waldhauser, J. Kovacs, and E. Reiter, *Exp. Gerontol.* 33, 759 (1998).
6. I. Haimov, *CNS Spectr.* 6, 502 (2001).
7. N. Buscemi, B. Vandermeer, N. Hooton, R. Pandya, L. Tjosvold, L. Hartling, S. Vohra, T. P. Klassen, and G. Baker, *BMJ* 332, 385 (2006).
8. C. Mallo, R. Zaidan, G. Galy, E. Vermeulen, J. Brun, G. Chazot, and B. Claustrat, *Eur. J. Clin. Pharmacol.* 38, 297 (1990).
9. R. L. DeMuro, A. N. Nafziger, D. E. Blask, A. M. Menhinick, and J. S. Bertino, *J. Clin. Pharmacol.* 40, 781 (2000).
10. N. Kanikkannan, S. Andega, S. Burton, R. J. Babu, and M. Singh, *Drug. Dev. Ind. Pharm.* 2, 205 (2004).
11. H. J. Oh, Y. K. Oh, and C. K. Kim, *Int. J. Pharm.* 212, 63 (2001).
12. K. K. Kandimalla, N. Kanikkannan, and M. Singh, *J. Control. Release* 61, 71 (1999).
13. M. R. Gasco, *Pharm. Technol. Eur.* 13, 32 (2001).
14. A. Bargoni, R. Cavalli, O. Caputo, A. Fundarò, M. R. Gasco, and G. P. Zara, *Pharm. Res.* 15, 745 (1996).
15. R. Cavalli, G. P. Zara, O. Caputo, A. Bargoni, A. Fundarò, and M. R. Gasco, *Pharmacol. Res.* 42, 541 (2000).
16. A. Fundarò, R. Cavalli, A. Bargoni, D. Vighetto, G. P. Zara, and M. R. Gasco, *Pharmacol. Res.* 42, 337 (2000).
17. V. Podio, G. P. Zara, M. Carazzzone, R. Cavalli, and M. R. Gasco, *J. Pharm. Pharmacol.* 52, 1057 (2000).
18. G. P. Zara, R. Cavalli, A. Bargoni, A. Fundarò, D. Vighetto, and M. R. Gasco, *J. Drug Targeting* 10, 327 (2002).
19. R. Cavalli, M. R. Gasco, P. Chetoni, S. Burgalassi, and M. F. Saettone, *Int. J. Pharm.* 238, 241 (2002).
20. S. E. Cross and M. S. Roberts, *Curr. Drug Deliv.* 1, 81 (2004).
21. B. J. Thomas and B. C. Fennin, *Drug Discov. Today* 9, 697 (2004).

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